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IMMUNIZATION OF WHITE MICE TO ECTROMELIA WITH VACCINE VIRUS - II

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Abstract

1. Intraperitoneal booster inoculation of vaccine virus (strain "Ankara") renews immunization to induced intraperitoneal ectromelia infection. The protection of the booster shot becomes attenuated during the same interval of time as that of the initial vaccination, i.e. 12 to 26 weeks.

2. Intraperitoneal administration of appropriate doses of commercially available vaccine lymph and/or tissue-culture vaccine produces the same immunization to induced intraperitoneal ectromelia infection as the inoculum from vaccine (strain "Ankara") infected chorio-allantois membranes.

3. Intraperitoneal vaccination increases the survival rate of a stock of mice with latent ectromelia as against non-vaccinated controls of the same stock. Female mice develop a higher immunity than male animals.

4. In the experience of the authors covering 5 years, vaccination of all freshly received mice in an institute suppresses the occurrence of ectromelia. The amount of time and cost for intraperitoneal vaccination is minor in comparison to other vaccination methods and to the possible losses from intercurrent ectromelia infection.

Introduction

Latent ectromelia in a stock of mice can be activated at any time through unfavorable external influences and stresses (poor shipping conditions, induced infection, radiation, tumor implantation, acute and chronic toxicity tests, etc.) (Ref. 1, 7, 8, 9). Tests with such animals are unreliable and make prolonged tests practically impossible.

Since Ref. 2 and 3 demonstrated that close antigenic relations exist between the virus of ectromelia and that of vaccinia, successful immunization attempts of mice to ectromelia with vaccine virus have repeatedly been described. The methods of administration of the several authors were intradermal (Ref. 1, 4, 5, 7, 8, 9), intravenous (Ref. 3, 4), intranasal (Ref. 1, 4, 10) and intraperitoneal (Ref. 1, 4).

An earlier communication (Ref. 11) stated that full immunization to induced intraperitoneal ectromelia can be provided, in confirmed non-ectromelic NMRI mice, by intraperitoneal administration of vaccine virus (strain "Ankara") adapted to chorio-allantois membranes. It was shown that the degree of immunization is a function of the dose of inoculation and that full protection exists after 6 days but attenuates gradually over 5 months.

In continuation of these earlier investigations, the application in practice of intraperitoneal vaccination was further tested through booster shots for already vaccinated mice together with the suitability of commercially available vaccine lymph for immunization. The effect of intraperitoneal vaccination on mice with confirmed latent ectromelia was also investigated. The authors report on practical experience covering 5 years with intraperitoneal vaccination.

Material and Methods

1. Test Mice

Unless otherwise stated, mice of the NMRI strain from the non-ectromelic stock of the Federal Research Institute for Virus Diseases of Animals in Tubingen (colony stock) were utilized.

2. Vaccine Virus

For intraperitoneal immunization, we utilized the vaccine-virus strain 10-54 "Ankara" furnished by the Federal Research Institute in Tubingen adapted to the chorio-allantois membrane and prepared after the 52nd passage. The strain was available as a "standard suspension" whose preparation was described in Ref. 11. Investigation of the suitability of commercially available vaccine lymph for intraperitoneal immunization of white mice was a vaccinia inoculum of the Bavarian State Vaccine Institute in Munich (No. 50-59, Portion 3).

3. Ectromelia Virus

The ectromelia virus utilized for induced infection was isolated from liver and spleen of mice from a commercially available CFW breed with latent ectromelia. Preparation of the standard ectromelia-virus suspension has been described in Ref. 11

4. Method of Inoculation

The vaccine-virus standard suspension stored at - 25 to 30° C in ampoules and/or the commercial vaccine lymph was freshly diluted with buffered physiological sodium-chloride solution as required prior to each inoculation. After addition of 100 gamma streptomycin and 100 IE penicillin per ml, the mixture was left to stand at 37° C for 20 minutes and subsequently injected intraperitoneal in the mice at a dose of 0.2 ml/animal after prior disinfection of the point of entry with iodine-alcohol solution. For routine inoculation, a tissue-culture vaccine has been employed for the last 2 years (for details of procedure cf. subsection on tests, para. 4).

5. Incubation Eggs

Incubation eggs (white Leghorn) of the Zimmerman Farm in Wiesloch were utilized for culturing the vaccine virus on chorio-allantois membranes.

Tests

1. Booster Inoculation

The following tests were intended to determine whether intraperitoneal booster inoculation is tolerated by already vaccinated mice and/or whether such booster inoculation produces improved immunization to induced intraperitoneal ectromelia.

Six groups with the same number of male and female mice (15 and/or 20 males and females per group) were inoculated with 0.2 ml/animal intraperitoneal at a dilution of the vaccine-virus standard suspension of 10^{-1}. 36 weeks later, the animals from 3 of the groups were re-inoculated with the same dose whereas the mice of the remaining 3 groups were not. To test immunity of twice and of only once vaccinated mice, intraperitoneal infection with ectromelia (0.2 ml/animal diluted at 10^{-5}) was induced at different intervals of time. In the mice vaccinated only once, infection was made 40, 48 and 62 weeks after the first vaccination. The animals inoculated twice were infected 4, 12 and 26 weeks after revaccination.

Virulence of the ectromelia-virus suspension employed for infection at various points in time was tested on non-vaccinated and non-ectromelic mice as controls. Post observation time was 6 weeks each (cf. Table 1).

Table 1

Survival Rate of Mice Infected with Ectromelia Virus

a— Infektion mit E-Virus (0,2 ml einer Verdünnung 10^{-5} i.p.)	tot / insgesamt b— ♂	c— ♀
d— 1. Einmal vaccinierte Mause		
a) 40 Wo. nach 1. Vaccination	2/20	3/20
b) 48 Wo. nach 1. Vaccination	13/20	4/20
c) 62 Wo. nach 1. Vaccination	14/15	3/15
e— 2. Revaccinierte Mause		
a) 40 Wo. nach 1. Vaccination / 4 Wo. nach 2. Vaccination	0/20	0/20
b) 48 Wo. nach 1. Vaccination / 12 Wo. nach 2. Vaccination	3/19	0/20
c) 62 Wo. nach 1. Vaccination / 26 Wo. nach 2. Vaccination	7/15	3/15
f— 3. Infektionskontrollen		
a)	18/20	18/20
b) nicht vacciniert	20/20	20/20
c)	15/15	13/15

Key:
a. 0.2 ml diluted at 10^{-5} of E-virus
b. male
c. female
d. 1. once vaccinated mice
   a) 40 weeks after first vaccination
   b) 48 weeks after first vaccination
   c) 62 weeks after first vaccination
e. 2. twice vaccinated mice
   a) 40 weeks after first vaccination
      4 weeks after 2nd vaccination
   b) 48 weeks after first vaccination
      12 weeks after 2nd vaccination
   c) 62 weeks after first vaccination
      26 weeks after 2nd vaccination
f. 3. infection controls
   a), b), c) not vaccinated

If mice were infected intraperitoneal with ectromelia virus (0.2 ml diluted at 10^{-5} per animal) 40 weeks after single vaccination, 2 (10%) of the male and 3(15%) of the female animals died. With the same dose for the non-vaccinated controls, 18 (90%) of both male and female mice died. Among the revaccinated mice (40 weeks after the first and 4 weeks after the 2nd vaccination), 20 male and 20 female animals survived.

48 weeks after single vaccination, 13(65%) of 20 male and 4(20% of 20 female mice similarly infected died. Under the same conditions, all 20 male and 20 female non-vaccinated control animals died. Among the mice revaccinated 40 weeks after the first and 12 weeks after the second vaccination, 3 (16%) of 19 males died whereas all 20 female animals survived.

After infection intraperitoneal with ectromelia virus 62 weeks after single vaccination, 14(93%) of 15 male and 4(26%) of 15 female animals died.

Among the non-vaccinated controls, all 15(100%) male and 13(88%) of 15 female mice died under the same conditions. However, of the revaccinated animals (62 weeks after the first and 26 weeks after the 2nd vaccination), only 7(46%) of 15 male and 3(20%) of 15 female mice died.

2. Investigations on Suitability of Commercial Vaccine Lymph for Immunization of White Mice to Ectromelia

In order to be able to carry out the vaccination of mice against ectromelia independent of the equipment required for continued cultivation of vaccine virus on chorio-allantois membrane in each laboratory, we investigated whether intraperitoneal inoculation of white mice with a commercial vaccine lymph can be made as successfully as with the inoculum utilized in our previous tests which was prepared from vaccine-infected chorio-allantois membranes.

Prior to carrying out the tests, the virulence of both infective agents was investigated comparatively on 10-day chicken embryos. For this purpose 0.2 ml each of dilutions of 10^{-2} to 10^{-6} of the commercial vaccine lymph and of the inoculum (standard suspension) prepared from chorio-allantois membranes infected with the vaccine virus (strain "Ankara") was placed on the chorio-allantois membrane of 10-day chicken embryos (11 and/or 12 embryos per dose) and the death rate and interval before death of the infected embryos determined. We also compared the extent of pox formation (+/++/+++; generalization) on the chorio-allantois membrane for the different doses of the 2 agents (cf. Table 2).

Table 2

Survival Rate of 10-Day Chicken Embryos after Infection of the Chorio-Allantois Membrane with Standard Suspensions of 2 Vaccine Virus (Pox Inoculum of the Bavarian State Vaccination Institute in Munich and Vaccine Virus (strain "Ankara").

a– Zahl der Embryonen	b– Infektions-Dosis 0,2 ml/Embryo der Verdünnungen	c– tot/insgesamt	d– + nach Tagen
e– Pockenlymphe der Bayer. Impfanstalt			
12	10^{-2}	12/12	3–5
11	10^{-3}	11/11	3–5
12	10^{-4}	12/12	4–7
11	10^{-5}	3/11	5–6
11	10^{-6}	1/11	7
f– Stammsuspension, Stamm „Ankara"			
11	10^{-5}	11/11	2–3
11	10^{-5}	11/11	2–3
12	10^{-4}	12/12	3–4
11	10^{-5}	11/11	3–5
12	10^{-6}	6/12	4–5

Key: a - number of embryos; b - infective dose; c - died/total; d - after x days; e - vaccine lymph; f - vaccine virus

Both strains of vaccine virus at doses of 0.2 ml diluted at 10^{-2} to 10^{-4} killed all embryos. However, the embryos inoculated with the vaccine virus strain "Ankara" died earlier than those infected with the vaccine lymph. 0.2 ml diluted at 10^{-5} of the "Ankara" strain caused death of all embryos within 3 to 5 days whereas infection with the vaccine lymph killed on 3 of 11 embryos within 5-6 days. 0.2 ml diluted at 10^{-6} of the "Ankara" strain still killed 6 of 12 embryos in 4 to 5 days whereas the vaccine lymph killed only 1 of 11 embryos after 7 days.

The pox found on the chorio-allantois membranes and produced by the 2 vaccine virus could not be definitely differentiated by number and appearance.

As expected, the virulence of the "Ankara" strain for chicken embryos consequently was somewhat higher than that of the vaccine lymph. In regard to number and appearance of the pox on the chorio-allantois membrane, microscopic examination was unable to determine any reliable difference between the 2 agents. The following comparative tests were carried out with the 2 agents:

a) Investigations on the Start of Immunity after Vaccination with the 2 Agents

Groups of 10 male and 10 female each were vaccinated intraperitoneal with 0.2 ml diluted at 10^{-1} of the vaccine lymph and/or the strain "Ankara" diluted at 10^{-1} and were then infected, after 3, 6 and 9 days, with dilutions at 10^{-1}, 10^{-3} and 10^{-5} (0.2 ml/animal) of the standard suspension of ectromelia virus and observed over a period of 8 weeks (cf. Table 3).

Both inoculums protected within 3 days after vaccination the greater part of the test animals against induced intraperitoneal ectromelia. Practically all mice inoculated with strain "Ankara" and/or the commercial vaccine lymph and infected, 6 or 9 days after inoculation, with 0.2 ml diluted at 10^{-3} and 10^{-3} survived. All non-vaccinated control animals died. Infection with 0.2 ml diluted at 10^{-5} of ectromelia virus was fatal only for a part of the non-vaccinated controls. The commercial vaccine lymph produced, under the test conditions described, the same result as the inoculum prepared from infected chorio-allantois membranes.

b) Investigations for Determination of the Inoculation Dose of the 2 Inoculums Required for Definite Protection against Induced Massive Intraperitoneal Ectromelia Infection

Groups of 10 male and 10 female mice each were inoculated intraperitoneal with 0.2 ml diluted at 10^{-1}, 10^{-2} and 10^{-3} of the strain "Ankara" and/or the commercial vaccine lymph. The same animals were infected intraperitoneal 9 days later with 0.2 ml diluted at 10^{-3} of our ectromelia virus (cf. Table 4).

Table 3

Comparison of Survival Rate of Mice Infected with Ectromelia

a–Tierzahl Geschlecht	b–Impfstoff Vaccine-Virus Dosis: 0,2 ml/Tier	c–Infektion E-Virus Dosis: 0,2 ml/Tier	d–Infektion nach Vaccination e–nach 3 Tg. tot	nach Tg.	e–nach 6 Tg. tot	nach Tg.	e–nach 9 Tg. tot	nach Tg.
10 m	f–Stamm „Ankara" Verd. 10^{-1}	10^{-1}	0		0		0	
10 w			4	9–13	0		0	
10 m		10^{-2}	0		0		1	11
10 w			0		0		0	
10 m		10^{-3}	0		0		0	
10 w			0		0		0	
10 m		—	0		0		0	
10 w			0		0		0	
10 m	g–Pocken-lymphe Bayer. Impfanstalt Verd. 10^{-1}	10^{-1}	0		0		0	
10 w			0		0		0	
10 m		10^{-2}	1	37	0		0	
10 w			1	53	0		0	
10 m		10^{-3}	0		1	30	0	
10 w			0		0		0	
10 m		—						
10 w			0		0		0	
Infektionskontrollen								
10 m		10^{-1}	10	7–14	10	6–10	10	6–12
10 w			10	7–12	10	6–13	10	6–12
10 m		10^{-2}	10	7–13	10	6–16	10	9–17
10 w			10	7–18	10	8–16	9	9–17
10 m		10^{-3}	4	9–16	2	10–15	1	10
10 w			3	10–16	2	10–12	0	

h–m = männlich
w = weiblich

Key: a – number of animals; b – inoculum; c – E-virus (dilution); d – infected after – 3 days – 6 days – 9 days; e – died – within days; f – strain "Ankara" diluted 10^{-1}; g – vaccine lymph diluted 10^{-1}; h – male – w = female

At a dose of 0.2 ml diluted at 10^{-1}, both inoculums protected all vaccinated mice against massive intraperitoneal ectromelia infection. Even after protective inoculation with 0.2 ml of the inoculums diluted at 10^{-2}, practically all mice were immune to the subsequent intraperitoneal ectromelia infection which was fatal for 95% of all non-vaccinated control animals. However, after inoculation of 0.2 ml of both inoculums diluted at 10^{-3}, 20 and/or 30% of the vaccinated mice still died after the same infection with ectromelia.

Table 4

Determination of Protective Dose against Induced Ectromelia Infection at Various Dilutions of Commercial Vaccine Lymph and of the Standard Suspension of Strain Ankara and Observed for 8 Weeks

a Tierzahl Geschlecht	b Impfstoff Vaccine-Virus	c Impfstoff-Dosis: 0,2 ml/Tier	d Infektion E-Virus 0,2 ml/Tier	e tot	f nach Tg.
10 m	g Stamm „Ankara"	10^{-1}	Verd. 10^{-8}	0	
10 w				0	
10 m		10^{-2}		1	12
10 w				0	
10 m		10^{-3}		4	11-18
10 w				2	11 u. 20
10 m	h Pocken-lymphe Bayer. Impfanstalt	10^{-1}	Verd. 10^{-8}	0	
10 w				0	
10 m		10^{-2}		0	
10 w				0	
10 m		10^{-3}		3	12-20
10 w				1	19
i Infektionskontrollen					
10 m	—		Verd. 10^{-8}	10	9-17
10 w				9	9-17

k m = männlich
w = weiblich

Key: a - number of animals; b - inoculum; c - 0.2 ml inoculum diluted at; d - E-virus 0.2 ml diluted at; e - died; f - within days; g - strain "Ankara"; h - vaccine lymph; i - control animals; k - m = male - w = female

3. Inoculation Against Ectromelia of Mice with Latent Ectromelia

In order to determine the effectiveness of intraperitoneal vaccination in mice with latent ectromelia, we carried out tests on mice commercially obtained from a breed with latent ectromelia. The ectromelia virus utilized for induced infection in our preceding tests had been isolated from this breed.

From a shipment of 500 mice of this CFW-stock (250 male and 250 females, average weight 18-20 g), we formed 2 groups of 246 mice (123 male and 123 female) after an initial period of observation of 2 weeks (death rate 3/500). 1 group was kept without any treatment under normal cage conditions for 28 weeks and the death rate recorded. The second group was injected with 0.2 ml diluted at 10^{-1} of the "Ankara" vaccine virus after the initial period of observation and their death rate over 28 weeks was also recorded (cf. Fig. 1).

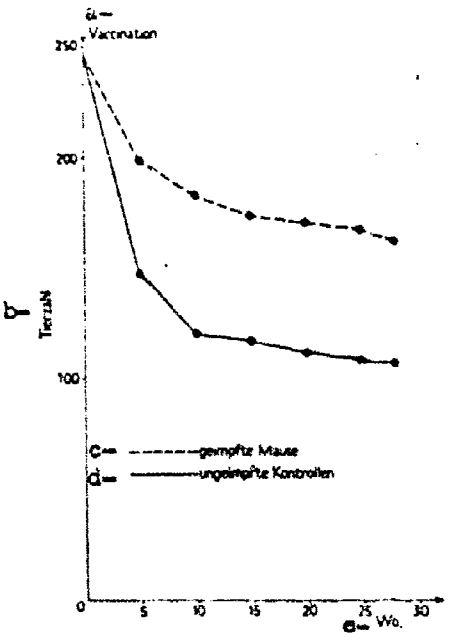

Fig. 1 Death Rate of Mice with Latent Ectromelia after Intraperitoneal Inoculation as Compared to Non-Vaccinated Mice.
a - vaccination; b - number of animals; c - inoculated mice;
d - non-inoculated controls; e - weeks

Among the non-vaccinated control animals with latent ectromelia, 126 (68 male and 58 female = 50%) died in the first 10 weeks of observation. Within 28 weeks, 139 mice (78 male and 60 female = 60%) of the animals had died.

Among the vaccinated mice, only 63 animals (51 male and 12 female = 25%) died within the first 10 weeks of observation. Within 28 weeks, only 83 animals (70 male and 13 female = 35%) died.

During obduction of the dead mice from both groups, particular attention was given to necrosis of liver and spleen. Among the non-vaccinated animals who died in the first 10 weeks, circumscribed necrosis of liver and spleen was observed more frequently than in the vaccinated mice. After this interval, the dead mice of both groups showed no macroscopically recognizable changes of liver and spleen. Histological examination was not made.

The gradient of the mortality curves and the obduction findings make it probable that an activation of the latent ectromelia took place at the start of the test which led to a generalized outbreak of the disease in the entire stock of animals.

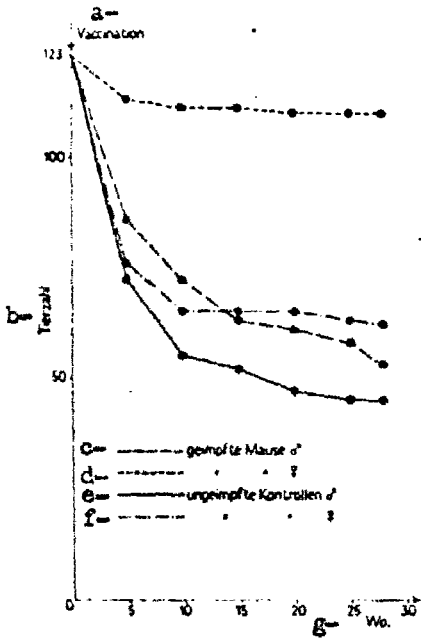

Fig. 2 Mortality Curves of Male and Female CFW Mice with Latent Ectromelia after Intraperitoneal Inoculation with Vaccine Virus in Comparison to Non-Vaccinated Mice.
a - vaccination; b - number of animals; c - vaccinated male mice; d - vaccinated female mice; e - non-vaccinated male controls; f - non-vaccinated female controls; g - weeks

In plotting the death rate by sex (cf. Fig. 2), no confirmed difference in regard to death rate and interval of death between male and female animals resulted from the non-vaccinated control animals of the stock of mice with latent ectromelia. In the vaccinated male mice, the death rate and interval before death was only slightly above that of the values found for non-vaccinated male controls. However, among the female mice about 90% of the vaccinated survived in comparison to about 50% of the non-vaccinated control animals. In order to determine the state of immunity of the mice surviving the 28-week test, the still living non-vaccinated 45 male and 62 female animals as well as the vaccinated 53 and 110 female mice were infected intraperitoneal with a massive dose of ectromelia virus (0.2 ml diluted at 10^{-2}).

Of the non-vaccinated mice surviving the ectromelia infection of the test, all 62 female animals survived the induced intraperitoneal ectromelia infection without signs of illness. Among the 45 male animals, 2(4.5%) died within 2 weeks. Among the surviving vaccinated 53 male and 110 female mice, 7 male (13%) and 16 female (14%) animals died within 2 weeks after the same intraperitoneal ectromelia infection.

4. Practical Experience with Intraperitoneal Vaccination

Between 1959 and 1964, all 95,700 mice purchased by our Institute were protected against ectromelia by intraperitoneal vaccination.

In the time from 1959 to early 1962, we utilized the inoculum prepared from chorio-allantois membranes infected with the "Ankara" vaccine virus as described in Ref. 11. Immediately prior to inoculation, the inoculum (standard suspension) was diluted 1:10 and 0.2 ml per animal injected intraperitoneal. Subsequently and until the middle of 1962, protective vaccination was carried out with the same inoculum but diluted 1:50. After it had been demonstrated that the commercial vaccine lymph (cf. above) furnished the same protection as the "Ankara" inoculum, we converted to the commercial vaccine lymph at 0.2 ml per animal in a dilution of 1:50. Since November 1962 until the end of 1964, we have been using for vaccination no longer the commercial vaccine lymph but a less expensive tissue-culture vaccine of the Bavarian State Vaccination Institute whose virus concentration, according to the vendor corresponds to about 1/10 of the commercial vaccine lymph (Ref. 6).

Protective inoculation against ectromelia with the tissue-culture vaccine is made by us presently as follows:

The sterile tissue-culture vaccine of the Bavarian State Vaccination Institute is diluted 1:5 with physiological sodium-chloride solution under sterile precautions. The dilution of the virus suspension receives in addition of 100 gamma streptomycin and 100 IE penicillin per ml, is left to stand at 37° C for 20 minutes, and utilized subsequently for intraperitoneal vaccination. Mice weighing 16 g or more receive 0.2 ml intraperitoneal after previous disinfection of the skin with iodine-alcohol solution.

From our present observations, intraperitoneal vaccination of mice as described has no deleterious effects. After a quarantine of 10 days, the animals are vaccinated. Since then, prolonged tests with mice over the entire lifetime of the animals have become possible in contrast to earlier efforts. The mean survival time determined from 50 male and 50 female vaccinated mice has been determined by others as 764 and/or 744 days under individual caging of the animals and feeding with "Lab-Blox" diet (Allied Mills Company, Chicago) and water at will. Since introduction of protective inoculation, ectromelia has no longer been observed among the stock of mice of our institute so that, up to the present, a booster vaccination of the mice during prolonged tests has not been necessary.

Discussion

Protective intraperitoneal vaccination of white mice against ectromelia with active vaccine virus has been satisfactory in practice and confirms earlier investigations. Our own experience covering 5 years on some 100,000 mice indicate that vaccination is well tolerated and makes it possible to

prevent outbreak of ectromelia in a stock of mice. The time required for intraperitoneal vaccination is very little in comparison to intradermal, intranasal or intravenous administration so that the injection of more substantial vaccine-virus amounts in order to achieve immunization is of no significance. Costs for intraperitoneal vaccination also are negligible in view of the possible losses from an intercurrent ectromelia. The immunity produced by intraperitoneal vaccination of mice is entirely comparable to that produced by intradermal introduction (Ref. 1, 5, 9, 10).

The intraperitoneal booster shot is also well tolerated and renews full immunity to ectromelia in the same manner as an intradermal booster shot. As in earlier investigations (Ref. 11), a higher immunity is observed among females than among male mice. Both after single as well as after repeated intraperitoneal vaccination, immunity becomes attenuated, however, for all animals over a period of 12 to 26 weeks in the same manner as described in Ref. 9 also for single and repeated intradermal vaccination. Of importance for the application of the intraperitoneal vaccination method in practice is the fact that commercial vaccine lymph can be utilized with the same satisfactory success as in the experience with intradermal vaccination (Ref. 1, 4, 5, 7, 8, 9). The present investigations show further that the same protection against induced ectromelia is achieved with commercial vaccine lymph and/or a tissue-culture vaccine as with the inoculum prepared from vaccine-infected chorio-allantois membranes. Any laboratory therefore has the possibility of vaccinating test mice intraperitoneal against ectromelia with commercial vaccine lymph without the necessity for cultivating the vaccine virus on the chorio-allentois membrane of incubated chicken embryos. The intraperitoneal vaccination method moreover has the advantage that an external contamination by the virulent vaccine virus occurring during intradermal administration in the skin of the tail of the base is prevented.

Mice with latent ectromelia also tolerate intraperitoneal vaccination well which confirms the experiences made with intradermal vaccination (Ref. 5, 9). If intraperitoneal vaccination is carried out during activation of latent ectromelia, immunization can still be acquired by a part of the animals, primarily female mice whereas vaccinated male mice die almost to the same extent as the non-vaccinated controls from the acute ectromelia. The immunity acquired after surviving a natural ectromelia infection appears to be greater, from present investigative findings, against artificial infection induced 4 to 6 months later than the immunity of the corresponding mice immunized by intraperitoneal vaccination.

For the suppression of ectromelia latent in a stock of mice as well as for the protection against ectromelia of a stock of mice, general intraperitoneal inoculation with active vaccine virus represents, from the available investigative findings, a simple, inexpensive and effective method in comparison to intracutaneous, intranasal and intravenous vaccination methods. Vaccination against ectromelia will continue to be necessary until a sufficient number of mice from non-ectromelic breeds (SPF-breeds) are available which can be reliably protected against infection by ectromelia also in the individual laboratories.

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